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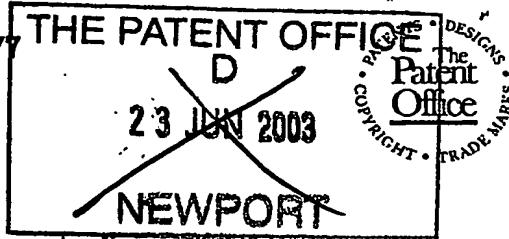
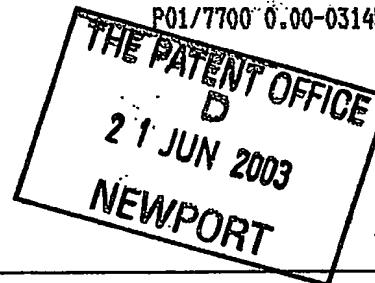
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IMPERIAL SCIENCE PARK
NEWPORT
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WALES

8658320001

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

LEUKOCYTE CELL BANKS

5. Name of your agent (*if you have one*)

"Address for service" in the United Kingdom to which all correspondence should be sent
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DR. VINCENT A. PRICE
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Description

2

Claim(s)

Abstract

Drawing(s)

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DUPPLICATE

LEUKOCYTE CELL BANKS

5

Field of the Invention

The invention relates to a novel form of leukapheresis (isolated leukapheresis), to processes and apparatus for carrying out isolated leukapheresis, to leukocyte cell banks created thereby and to various forms of therapy based thereon.

10

Background to the Invention

Cell banks

15

Cell banking is a service industry in which live cells are stored for later use. It has been practised for decades, and is exemplified by the storage of bovine sperm cells for the artificial insemination of cows.

20

With the technical advances that are being made in bio-medical research and tissue engineering, it is being recognized that many possibilities may exist for the use of human stem cells for various replacement therapies. These developments have led to a growing demand for facilities where stem cells of individuals can be isolated, cryo-preserved, and stored for later (autologous) use. For example, the desirability of storing the cord blood stem cells of newborns is becoming increasingly recognized and as a result there is a rapidly increasing number of deposits of such stem cells in private cell banks.

Leukapheresis

30

Leukapheresis is a specific form of apheresis which involves the selective separation and removal of leucocytes from withdrawn blood, the remainder of the blood then being retransfused into the donor. During leukapheresis, the removed blood is passed through a cell separation device which separates nucleated white

blood cells from red blood cells and plasma outside the body. The red blood cells and plasma are returned to the individual, as part of the separation process. The process is continuous with blood being removed and returned almost simultaneously after various extractions have been performed. Leukapheresis 5 therefore makes it possible to remove and return the entire blood volume of the individual several times over and separate out and keep large numbers of white cells without detriment to the individual.

The current separation systems, such as the Cobe® system (Cobe BCT, 10 Lakewood, CO. USA), are capable of extracting between 40% and 50% of the total white cells in the whole blood that passes through the separator, and the flow rate can be 40-60 ml or more per minute.

Use of leukapheresis in the creation of leukocyte cell banks

15 Leukapheresis has recently been proposed as a means for creating lymphocyte cell banks (see WO 00/29551 and WO 01/88099). However, the use of leukapheresis for the generation of comprehensive lymphocyte cell banks on a commercial basis is limited by donor convenience and donor comfort. For instance, donors typically 20 have only a certain amount of time which may be committed to visiting a blood component collection facility for donation. Consequently, once at the collection facility the amount of the donor's time which is actually spent collecting blood components is an important factor. However, leukapheresis generally takes between 2 and 4 hours (several times longer than the time required for the 25 donation of a unit of blood). This is in turn related to donor comfort: many view the actual collection procedure as daunting and of potential risk in that at least one (and usually two) access needles (for flow and return of the blood) must be in the donor throughout the procedure.

30 There is therefore a need for a process for producing leukocyte cell banks that avoids the problems associated with the use of leukapheresis and which is more convenient (and less uncomfortable) for donors, thereby making feasible the creation of comprehensive leukocyte cell banks for use in autotransplantation.

Summary of the Invention

- 5 The present invention is based, at least in part, on the discovery that blood samples collected in the usual way from a donor can provide a convenient source of leukocytes for banking if processed using commercially available leukapheresis devices: there is no need for the devices to be operated with the donor "in-line".
- 10 The leukocyte preparations produced by such an "isolated leukapheresis" process can be used for autotransplantation in the treatment of a variety of diseases, either directly or after various treatments have been performed on the leukocytes.

Thus, in a first aspect the invention provides isolated leukapheresis, in which the 15 leukapheresis device is not in fluid communication with the individual providing the blood sample and/or the remainder of the blood in the sample is not retransfused into the individual.

According to a second aspect of the present invention there is provided the use of 20 a leukapheresis device for selectively separating and removing leukocytes from a blood sample which is not in fluid communication with the blood of the donor from which it originated (i.e. is an isolated blood sample as herein defined).

According to a third aspect of the present invention there is provided a process for 25 producing a leukocyte composition for autotransplantation comprising the steps of: (a) providing an isolated blood sample from a donor individual and (b) selectively separating and collecting leukocytes from the sample using a leukapheresis device.

30 According to a fourth aspect of the invention there is provided a process for producing a leukocyte composition for restorative autotransplantation comprising the steps of: (a) providing an isolated blood sample from a donor individual; (b) selectively separating and collecting leukocytes from the sample using a leukapheresis device; (c) rendering the collected leukocytes dormant (e.g. by

cryogenic preservation); and optionally (d) revitalizing the dormant leukocytes (e.g. by thawing and/or dilution).

According to a fifth aspect of the invention there is provided a process for
5 producing a leukocyte composition for remedial autotransplantation comprising
the steps of: (a) providing an isolated blood sample from a donor individual; (b)
selectively separating and collecting leukocytes from the sample using a
leukapheresis device; (c) treating the collected leukocytes; and optionally (d)
rendering the treated leukocytes dormant (e.g. by cryogenic preservation).

10

In a sixth aspect the invention provides a process for producing a leukocyte cell
bank wherein the steps of: (a) providing an isolated blood sample from a donor
individual; (b) selectively separating and collecting leukocytes from the sample
using a leukapheresis device; and (c) rendering the collected leukocytes dormant
15 (e.g. by cryogenic preservation) are applied iteratively to a series of blood samples
from different donor individuals to produce a plurality of dormant (e.g.
cryogenically preserved) leukocyte compositions, the process further comprising
the step of: (d) retrievably depositing the dormant leukocytes for later
autotransplantation.

20

In a seventh aspect the invention provides a system (e.g. a closed system) for
collecting an isolated blood sample from an individual comprising a sample vessel
and a leukapheresis tubing set.

25 According to an eighth aspect the invention provides apparatus for selectively
separating and removing leukocytes from an isolated blood sample from an
individual comprising a leukapheresis device loaded with the collection system of
the invention.

30 The invention also contemplates a leukocyte composition and a leukocyte cell
bank obtainable (or obtained) by the process of the invention.

Also contemplated are various therapeutic uses for the processes, systems,
apparatus, compositions and banks of the invention. Accordingly, the invention

contemplates the leukocyte composition of the invention for use in therapy, for example in autotransplantation (e.g. in restorative or remedial autotransplantation).

5

Detailed Description of the Invention

10

I. Definitions

Where used herein and unless specifically indicated otherwise, the following terms are intended to have the following meanings in addition to any broader (or narrower) meanings the terms might enjoy in the art:

15

The term *leukapheresis* is a term of art used herein to define a procedure involving the selective separation and removal of leukocytes from the withdrawn blood of a donor, the remainder of the blood then being retransfused into the donor.

20

A *leukapheresis device* is a term of art defining any device capable of performing leukapheresis, irrespective of the means employed in the device to separate and remove the leukocytes.

25

The term *isolated leukapheresis* is used herein to define a novel form of leukapheresis which is performed on an *isolated* blood sample.

Similarly, the term *isolated apheresis* is used herein to define a novel form of apheresis which is performed on an isolated blood sample.

30

The term *isolated blood sample* is used herein to define a blood sample which is not in fluid communication with the blood of the donor from which it originated. Thus, in the process of isolated leukapheresis which is applied to isolated blood samples, the leukapheresis device is not in fluid communication with the

individual providing the blood sample and/or the remainder of the blood in the sample is not retransfused into the individual.

The term *autotransplantation* is used herein to define autologous transplantation (autogeneic or self-to-self transplantation), wherein the term *autologous* is used to indicate that the transplantation is to the same organism (i.e. the same individual) from which the cellular material (e.g. leukocytes) was removed. As used herein, *transplantation* defines any procedure involving the introduction of cellular material (e.g. leukocytes) into an organism, and so any form of transplantation or grafting known in the art is encompassed.

The term *dormancy* is used herein to define any state of suspended animation or stasis, and procedures for achieving this are well known in the art, as described below. Any of the known procedures may be used, including cryopreservation.

Thus, the leukocytes may be held or maintained in a quiescent, inactive or non-proliferating state.

The term *healthy* is used herein in relation to an individual donor to indicate that the individual is not suffering from a leukocytic deficiency (as herein defined).

Thus, the term *healthy* as used herein encompasses *non-diseased* individual donors in a state in which the individual donor is not suffering from any disease or disorder, or is not manifesting any symptoms of said disease or disorder (i.e. is asymptomatic or is in a pre-clinical condition). In particular, term *healthy* as used herein encompasses individual donors not suffering from, or demonstrating symptoms of, the disease or disorder which it is subsequently intended to treat by the autotransplantation procedure.

II. Blood samples

30

The invention may be applied to any form of blood sample, provided that: (a) the sample is isolated in the sense defined above and (b) the sample comprises at least some leukocytes from the individual donor.

The blood sample may be subjected to various treatments *ex vivo* prior to use in the process of the invention. Typically, for example, the blood sample is chilled prior to use. Other treatments may include the addition of preservatives and/or anticoagulants.

5

The blood sample may also be treated *in vivo* prior to collection by administering various agents to the donor individual before or during sample collection.

- Examples of treatments (which may be applied *ex vivo* and/or *in vivo*) are
10 discussed in more detail in the section entitled "Leukocyte treatments", below.

It is generally preferable to sample at least 450-500 ml of blood from the individual donor, which is the equivalent of a unit of blood as provided by a blood donor for the UK blood transfusion service. If possible a number of samples (e.g.
15 several 450-500 ml samples) are taken over a period of time (e.g. over 2-3 weeks, preferably 2-3 months or over 6 months or a year, 2 or 3 years or more). One or more of these can then be divided or combined into a number of leukocyte cell bank deposits. The removal of a unit of blood is commonplace with over three million units of blood being taken, for allografting, from individuals annually in
20 the UK alone.

The blood removed is soon replaced and, therefore, multiple samplings of a unit of blood from an individual can be provided over a year, say 2-12 unit samplings if necessary, without detriment to the individual being sampled.

25

III. Selection of donor individuals

General considerations

30

Restorative autotransplantation is a form of therapy that might ultimately be indicated for any individual. Consequently, the invention may be usefully applied to the generation of comprehensive leukocyte cell banks covering as large a number of different individuals as possible in order that restorative

autotransplantation can be carried out in any of the represented individuals should the need arise.

It is therefore contemplated that the invention be applied as broadly as possible so
5 that a comprehensive leukocyte cell bank can be assembled. However, since the quality of the individual deposits will depend (at least to some extent) on the health status of the individual donor at the time of blood sample donation, it is preferred that the blood sample for use in the processes of the invention be taken from healthy individual donors.

10

Other factors also affect donor selection: for example, the blood sample for use in the processes of the invention may advantageously be obtained from individual donors when they are young, preferably in adolescence or early adulthood. In the case of humans, blood sampling (preferably multiple sampling) at the ages of
15 about 12 to 30, preferably 15 to 25 is preferred. Especially preferably, sampling is from the age of 16 or 17 upwards, for example in the age range 16 to 30, 17 to 30, or 18 to 30, or perhaps 18 to 35 or 40. It is thus preferred that the cells be obtained when the host organism is mature, or reaching maturity, but before the processes of ageing or senescence have significantly set in. In particular, it is
20 preferred and advantageous that the immune system of the host organism is mature or fully developed.

However, the obtention of cells outside these ranges is encompassed, and cells may be obtained at any post-natal life stage e.g. from juvenile host organisms e.g.
25 in mid-to late childhood, or even infants, or from older individuals.

Sampling from post-natal or older hosts allows multiple samples to be collected, thereby increasing the opportunity of storing sufficient number of cells. In addition sampling from juvenile or older hosts overcomes the ethical requirements
30 such as providing informed consent.

Sampling from adolescent or adult host organisms is preferred since the sampled cells, from blood in particular, will contain a greater proportion of valuable mature T-cells capable of recognising aberrant cell populations, such as cancer cells or

virally infected cells. Thus, when blood samples are used, it is advantageous that they are taken from an individual with a mature immune system (i.e. not foetal or neonatal).

- 5 Thus, the invention contemplates the use of blood samples collected from donor individuals at a stage when there is no direct prediction, suggestion, or suspicion that a particular disorder or disease may develop, for use against a future possible or unpredicted event, or an event which may occur simply by chance, rather than an anticipated or suspected or predicted illness or condition. Thus, in certain
10 embodiments of the invention, the donor individual is not predisposed to, or at risk from, any particular disease or disorder e.g. not exhibiting any symptoms or manifestations predictive of a subsequent disease or disorder. Likewise, the host organism is preferably not suffering from any injuries or damage which may give rise to an anticipated or expected condition.

15

- Indeed, for certain applications (for example, the generation of leukocyte cell banks for subsequent restorative autotransplantation) it is preferred that the blood sample for use in the invention be obtained from the donor individual before any disease or disorder develops or manifests itself, and more preferably when the
20 host organism is in general good health, and preferably not immunocompromised in any way. In such embodiments it is particularly advantageous to sample the blood from donor individuals at a time when the organism has not previously exhibited symptoms of or presented with or been diagnosed as suffering from the disease or disorder which is subsequently to be treated, i.e. when the host
25 organism is healthy and not "in remission" e.g. not in a state of partial or full recovery from the leukocyte deficiency to be treated.

Predisposed donor individuals

- 30 Advances in therapy continue to be made, and our greater understanding of disease processes helps us to modify and refocus our therapeutic approaches to alleviate disease and suffering. Such understanding has been greatly advanced by technological improvements in the field of molecular biology. We are now in a position to follow the pathogenesis of diseases at a molecular level, and recognize

the importance of an individual's genetic make-up in predisposing them to certain diseases. For example, we are aware that some individuals, because of their genetic composition, are prone to certain diseases.

- 5 Many of the diseases to which certain individuals can be predisposed are leukocyte deficiencies, which term is used herein to indicate a condition in which the administration of autologous leukocytes is indicated. Such conditions therefore include those in which an individual has acquired a disease, infection or condition involving leukocyte dysfunction or a disease, infection or condition in
10 which the augmentation or stimulation of endogenous leukocyte activity is indicated. Detailed examples of particular leukocyte deficiencies are set out in the section entitled "Exemplary indications", below.

- 15 Through genetic testing, therefore, it is now possible to identify those individuals predisposed to a leukocyte deficiency (e.g. any of various forms of cancer, immune disorder or infection).

Furthermore, our knowledge of the body's immune system, and in particular the way in which it recognises and kills virally infected and tumour cells, continues to
20 advance. We now know that in order to elicit cell-mediated immunity, an offending cell (e. g. a virally infected or tumour cell) must co-present an HLA class I restricted tumour or viral epitope with danger signals such as GM-CSF and/or TNF-alpha, so that the antigen presenting cells (APC) of the immune system will express co-stimulatory signals such as B7 and IL-12 in conjunction
25 with antigen to the interacting cytotoxic T-lymphocyte (CTL) population. The co-presentation leads to the production of clones of both activated and memory cells (for review see Nature Medicine Vaccine Supplement 4 (1998) 525). In the absence of these additional signals, HLA-I antigen-restricted T-cells which recognise offending cells are processed for destruction or desensitization (a bodily
30 process presumably put into place to avoid the development of e.g. autoimmune disease). The induction of such tolerance is because of either ignorance, anergy or physical deletion (Cold Spring Harbour Symp Quant Biol 2 (1989) 807; Nature 342 (1989) 564; Cell 65 (1991) 305; Nature Med 4 (1998) 525).

It is now clear that tumour cells do not automatically co-present danger and/or co-stimulatory signals. Hence, the spawning of a tumour may lead to eradication of the very cells that provide cell-mediated immunity against the tumour. A patient presenting with a cancer, leukaemia/lymphoma or sarcoma etc, therefore, may
5 have already removed their innate ability to destroy the tumour, by default.

However, if the required T lymphocytes, or a sample thereof, were removed from the patient prior to the onset of proliferative disease, the relevant T-cell population could now be returned to the patient, after the necessary co-stimulation of the T-
10 cells, so as to alleviate disease. Co-stimulation may be provided at the same time as the cells are returned to the patient, or after they are returned through further treatment (s) of the patient, or without stimulation other than that naturally produced by the patient. Activation/stimulation of the cells may also initially be induced *in vitro* prior to reinfusion.
15

The present invention therefore finds particular application in the case of individuals predisposed to the development of a leukocyte deficiency. It therefore represents a means for removing leukocytes from a healthy donor individual for subsequent transplantation to that same individual in a subsequent autologous
20 (autogeneic) transplantation procedure, when the need or desire to do so arises. Although the predisposed individual may never receive the cells because no disease to be treated by this method ever occurs, the invention nevertheless may be used to provide some form of insurance against the heightened risk of a leukocyte deficiency arising in the individual.

25 Similarly, individuals with no diagnosed predisposition may choose to provide samples for incorporation into the leukocyte cell bank of the invention for prospective use by themselves prior to travelling abroad. Such use might include for the treatment of infections contracted whilst abroad.

30 In addition, it is well recognized that the ageing process makes individuals more susceptible to disease. The basis for the susceptibility appears to be in the loss of immune function resulting from a significant decrease in T and B cell numbers/activity during ageing (Mech Ageing & Dev 91 (1996) 219; Science 273

(1996) 70; Mech Ageing & Dev 96 (1997) 1). Disease susceptibility is particularly pertinent when elderly patients are subjected to e.g. surgery in a hospital environment, where they are prone to opportunistic infections with serious or even fatal consequences. Blood samples taken from such individuals much earlier in
5 life and processed according to the invention for inclusion in a leukocyte cell bank could provide the opportunity for restorative autotransplantation in such circumstances.

Such an approach could be used more broadly to provide for a method of
10 augmenting the patient's immune system after surgery in order to lessen the likelihood of post-operative complications caused by opportunistic infections. The invention, therefore, could be used as a prophylactic therapy, e.g. for elderly patients when they are more susceptible to disease.

15

IV. Leukocytes

The invention contemplates the use of isolated leukapheresis to separate and collect leukocytes from a blood sample. It will be appreciated that the separation
20 and/or removal of leukocytes from the blood sample during leukapheresis need not be absolute. Rather, the removal and/or separation of a fraction of the total leukocytes present in the sample is sufficient in most circumstances. Those skilled in the art will readily be able to determine the appropriate size of the fraction to be removed, which will vary *inter alia* according to the use to which
25 the isolated leukocytes are to be put, the size of the sample, the status of the donor, the nature of the leukocytes and the particular leukapheresis device employed.

The leukocytes collected in the processes of the invention are to some degree
30 isolated from the original blood sample. The term *isolated* is used here to indicate that the isolated leukocytes exist in a physical milieu distinct from that in which they occur *in vivo* and does not imply any particular degree of purity. Indeed, the absolute level of purity is not critical, and those skilled in the art can readily

determine appropriate levels of purity according to the use to which the leukocytes are to be put.

The separation and collection of the leukocytes in the processes of the invention
5 also does not necessarily imply that any particular class or type of leukocyte is preferentially separated and collected. Rather, the leukocytes of the invention include any white blood cell, including granulocytes, lymphocytes and monocytes.

Granulocytes include myelocytes, basophils, eosinophils and neutrophils.

10 Lymphocytes include B, T lymphocytes and natural killer cells. Monocytes include mononuclear phagocytes and other macrophages.

However, in some embodiments the leukocytes which are separated and collected preferably comprise one or more specific leukocyte cell types. A preferred cell
15 type is the lymphocyte, especially a T-lymphocyte (T-cell). Mature T-lymphocytes are particularly preferred.

Since the total mature T-cell number per litre of blood ranges between $1-2.5 \times 10^9$ for humans, a 100 ml sample of blood typically contains $1-2.5 \times 10^8$ mature T-cells and this is generally sufficient to provide an adequate representation of the entire mature human T-cell population for the beneficial effect. However, depending on the fraction of total leukocytes separated and collected by the leukapheresis device and the efficiency of any revitalizing technique employed, preferably at least 100 ml, 115 ml, 200 ml or 300 ml and even more preferably in excess of 400 or 500 ml of blood sample is used in order to obtain the appropriate number of mature T-cells to support a beneficial therapeutic effect for return to the individual if and when they become ill.
25

Standard techniques are known in the art which permit selection of particular subpopulations of lymphocytes from a sample comprising a mixed population of lymphocytes. Examples of such subpopulations are CD3⁺, CD8⁺, CD4⁺ and CD16/56⁺ (natural killer) T cells and CD19⁺ B cells. For example, any one or any mixture or combination of such subpopulations of T cells can be used in the methods, uses and compositions of the invention, and they are readily obtained by
30

means of well known methods such as FACS (Fluorescence Activated Cell Sorting) and haemocytometry systems.

The invention also finds broader utility, and instead of leukapheresis any other
5 form of isolated apheresis may be employed. Thus, the invention contemplates a process of isolated apheresis for the production of a stem cell composition (and stem cell banks generated thereby). The invention may therefore be applied to stem or progenitor cells, including both pluripotential stem cells and stem or progenitor cells already committed to a particular path or paths of differentiation.

10

V. Leukocyte treatments

The leukocytes may be subjected to various treatments. Such treatments may, for
15 example, result in expansion of some or all of the representative cell subsets, improve the long-term viability of the leukocytes during the dormancy period, improve their therapeutic potency, remedy a deficiency or defect exhibited by some or all of the leukocytes (as is the case, for example, in remedial autotransplantation therapeutic modalities) and/or render their subsequent use in
20 autotransplantation safer.

The treatments can be carried out before or after the leukocytes are rendered dormant (and before or after autotransplantation is carried out). Moreover, the treatments may be applied after the blood sample is taken (i.e. be carried out *ex vivo*) either prior to rendering the cells dormant or after revitalization. For
25 example, treatment of the leukocytes may be effected by co administration of a separate composition, sequentially or simultaneously with the leukocyte composition, during autotransplantation. Treatment of the leukocytes can be effected immediately prior to autotransplantation.

30

Alternatively (or in addition) the treatments may be applied to the leukocytes while still *in vivo* prior to blood sampling by the administration of e.g. growth factors or cytokines (see below).

Exemplary pre-transplantation treatments may include various genetic modifications, such as the incorporation of a negative selection marker (as described, for example, in W096/14401, the content of which is incorporated herein by reference). Such treatment permits ablation of the leukocytes after 5 transplantation or titration of dose versus response. Other genetic interventions may include regulating or modifying the expression of one or more genes (e.g. increasing or decreasing gene expression), inactivating one or more genes, gene replacement and/or the expression of one or more heterologous genes). Other genetic modifications include the targeting of particular T-cells (as described in 10 W096/15238, the content of which is incorporated herein by reference), and the modification of the T-cell receptor repertoire/expression with antibodies to make T-cell chimaeras.

Other treatments contemplated by the invention include the exposure of the 15 leukocytes with one or more stimulatory molecules, for example antigens (e.g. cancer or viral antigens), antibodies, T cell recognition epitopes, peptides, blood factors, hormones, growth factors or cytokines or combinations thereof.

For example, the leukocytes may be treated *in vitro* (or *in vivo* prior to blood 20 sampling) with antigens (e.g. cancer or viral antigens), antibodies, T cell recognition epitopes, peptides, blood factors, hormones, growth factors or cytokines or combinations thereof. The stimulatory molecules may be synthetic, recombinant or may be purified or isolated from the human or animal body. Particularly useful in this respect are stimulatory molecules selected from IFN-alpha, IFN-beta, IFN-gamma, II-1a, II-1b, II-2, II-3, II-4, II-5, II-6, II-7, II-8, II-9, 25 II-10, II-11, II-12, II-13, II-14, II-15, GM-CSF, M-CSF, G-CSF, LT and combinations of two or more of the foregoing. Such treatments may modify the growth and/or activity and/or state of differentiation of the leukocytes, and/or may serve to separate or selectively isolate or enrich desired leukocyte cell types or to 30 purge unwanted cells.

Recent advances have been made in the way cells may be obtained for subsequent autotransplantation. For example, investigations into the agents which regulate haematopoiesis have led to the isolation of a series of factors that influence the

— proliferation and differentiation of lymphocytes. These agents include the cytokines (such as the interleukin series IL-1 to IL-18 and the leukotrienes) and growth factors such as the TNF's, the TGF's, FGF's, EGF's, GM-CSF, G-CSF and others. A number of these factors are now available commercially for clinical use,
5 and some have been shown to increase substantially the number of lymphocytic cells and, in particular, immature T-lymphocytes in the peripheral blood. Their administration to the donor individual prior to blood sampling permits the quantity and/or quality (in terms of the number and nature of leukocyte subtypes present) to be controlled and makes it possible to recover large quantities of the cells of
10 interest. e.g. immature T-lymphocytes, directly from the donor individuals peripheral blood sample without the need to sample the marrow.

Other pre-transplantation treatments include culture of the leukocytes (or a sub-population thereof). For example, the leukocytes may be cultured to increase cell
15 numbers. For example, the cells may be passaged, according to methods well known in the art. Such culturing may be carried out before or after the leukocytes are rendered dormant, or both before and after dormancy is induced.

Thus, in the case where the leukocytes include T-cells, the T-cells may be co-stimulated prior to transplantation and/or exposed to tumour antigens (optionally together with co-stimulatory factors) prior to autotransplantation.
20

VI. Leukapheresis devices

25 Many different types of leukapheresis devices are presently commercially available. Such devices usually comprise at least three separate elements: (1) a *separation device* (e.g. comprising a membrane or centrifuge rotor, which provides the forces for separating the leukocytes from the various other blood components: (2) one or more *pumps* for conveying the blood sample to the separation device, for removing the separated leukocytes and for maintaining the forces necessary for transfusion and retransfusion, and (3) a (normally disposable) *tubing set* which holds the blood and its various fractions in a particular geometry within the separation device, defines fixed channels through which the blood

flows in a circuit from the donor, through the leukapheresis device and back to the donor as well as vessels (usually bags) for the collection of the separated leukocytes and/or other blood fractions or fluids.

- 5 Any of a wide variety of commercially available leukapheresis devices may be used according to the present invention. The particular way in which the leukapheresis device is operated will depend on a number of factors, including the nature of the separation device (e.g. centrifuge, filter etc.), the type of leukocyte sample required, the volume of the blood sample to be processed, the identity and
- 10 status of the donor individual, the ultimate use to which the leukocyte composition is to be put and the nature of any treatments applied to the blood sample prior to processing according to the invention. Thus, those skilled in the art will readily be able to establish the appropriate operational parameters.
- 15 Preferably, however, the leukapheresis device is selected to minimize the need for operator intervention and/or training. Commercially available leukapheresis systems vary in the time and/or expertise required of an individual to prepare and operate it. For instance, reducing the time required by the operator to load and unload the tube set, as well as the complexity of these actions, can increase
- 20 productivity and/or reduce the potential for operator error. Moreover, reducing the dependency of the system on the operator may lead to reductions in operator errors and/or to reductions in the credentials desired/required for the operators of these systems.
- 25 Performance-related factors are also relevant, and may be judged *inter alia* in terms of the "collection efficiency" of the leukapheresis system. The "collection efficiency" of a system may of course be gauged in a variety of ways, such as by the size of the fraction of leukocytes collected in relation to the total leukocytes present in the sample. Performance may also be evaluated based upon the effect
- 30 which the leukapheresis procedure has on the various blood component types. For instance, it is desirable to minimize the adverse effects on at least the leukocytes of the apheresis procedure. It may also be desirable to reduce platelet activation, in order to avoid degeneration in sample quality during processing.

Particularly preferred is the Cobe® system (Cobe BCT, Lakewood, CO, USA).

VII. Collection systems for use in the invention

5

The systems for collecting an isolated blood sample from an individual for use according to the invention may comprise a sample vessel (for collecting and containing the blood sample) together with a leukapheresis tubing set.

10 The term *leukapheresis tubing set* is used herein to define a tubing set as described in the preceding section. The tubing set may comprise a *blood processing vessel* within which the leukocytes are subjected to separation forces in the separation device.

15 In the case of tubing sets for use with leukapheresis devices which comprise a centrifuge-type separation device (as described in the preceding section), the blood processing vessel may comprise a *centrifuge loop* which defines a vessel within which the blood is subjected to centrifugal separation forces when loaded into the centrifuge rotor of the separation device.

20

The systems for use according to the invention may be closed, functionally closed, or open.

25 As used herein the term *closed system*, as applied to a leukapheresis tubing set, is used to define tubing sets which are sterile and isolated from the outside environment by aseptic barrier(s) and in which all components are fully integral, being attached and/or assembled at the manufacturing site.

30 As used herein the term *functionally closed system*, as applied to a leukapheresis tubing set, is used to define tubing sets which are assembled at the device manufacturing site and which use sterile barrier filters (e.g. 0.22 micron filters) for the attachment by the end user of solutions and sterile connecting devices for filters.

As used herein the term *open system*, as applied to a leukapheresis tubing set, is used to define tubing sets which are only partially assembled at the device manufacturing site and then customized by the end user.

- 5 Preferably, the system further comprises one or more (e.g. three) leukocyte collection vessel(s). Three or more collection vessels are preferred, so that there is a degree of redundancy in the samples and also to facilitate the creation of cell banks with duplicate/triplicate samples. This permits more flexible autotransplantation regimes.

10

- The system also conveniently comprises a vessel for residual blood from which the leukocytes have been removed. This residual blood may prove to be of utility in other therapeutic paradigms, such as in an allogeneous setting. A needle or cannula may also be incorporated for conducting a blood sample from the individual into the sample vessel.

15

The various vessels conveniently take the form of flexible, transparent bags. Some (or all) of the tubing is also conveniently formed of flexible, transparent material (e.g. plastics).

20

VIII. Induction of dormancy

Any suitable means may be employed for inducing dormancy.

25

According to a preferred cryopreservation procedure, the cells are frozen preferably to a temperature below -160°C. A particularly preferred means of achieving dormancy is to freeze the cells to the boiling point of helium (He), i.e. to about -269°C or below.

30

As described in Freshney's (Freshney's Tissue Culture of Animal Cells (Culture of Animal Cells: A Manual of Basic Technique, Wiley Liss, 1994)), the cells may be suspended in a suitable medium (e. g. containing 5-10% DMSO) and cooled at a controlled rate (e. g. 1°C per minute to -70°C, then into liquid/gas N₂). Such

conventional procedures may be adapted to cool the cells into He/N₂ mixtures or He. Alternative methods of achieving and/or maintaining cell dormancy include cooling to 4°C.

5

IX. Revitalization

Following dormancy, the cells are revitalised prior to use in transplantation.

- Again, this may be achieved in any convenient manner known in the art, and any
10 method of revitalising or reviving the cells may be used.

Conveniently, this may, for example, be achieved by thawing and/or diluting the cells. Techniques for revitalisation are well known in the art. Cells may be thawed by gentle agitation of the container holding the cells in water at 37°C,
15 followed by dilution of DMSO to 1% or below, e. g. with medium or serum.

Cells may be implanted immediately or after recovery in culture. Revitalisation is designed to re-establish the usefulness of the cells e.g. in prophylaxis or curative therapy.

20

X. Cell banking

The leukocyte compositions of the invention may be banked, thereby creating a
25 leukocyte cell bank. Preferably, the compositions are banked after the leukocytes have been rendered dormant (as described above).

Any suitable cell banking system may be employed, provided that the deposits are retrievable for autotransplantation. This implies the use of some form of labelling,
30 but this need not be in the form of a physical appendage to the individual deposits.

Thus, the leukocyte cell bank of the invention may comprise a plurality of cell storage units for storage of leukocyte compositions. Typically, such cell storage is effected by cryopreservation, but other storage techniques can also be employed.

The cell banks of the invention may further include a digital information unit for digitally storing information relating to the identity, location and medical history of the donor individual and/or the conditions associated with the particular deposit (for example relating to the date at which the blood sample was collected from the 5 donor individual, the processing conditions and details of any treatments applied to the leucocytes contained in the deposit).

10 The digital information unit preferably comprises at least one digital computer having sufficient digital storage capacity for storage of the potentially large amounts of information relating to each deposit.

15 The leukocyte cell bank of the invention preferably further comprises an arrangement for digital data retrieval interfaced with the digital information unit for retrieving selected information stored in the digital information unit. The data retrieval arrangement may be integrated with the digital computer. Remote access of the digital information *via* the telephone or the internet may also be provided and may permit rapid and convenient access of the information on a global basis.

20 XI. Medical applications

The invention finds application in all forms of therapy and prophylaxis in which the administration of (treated or untreated) autologous leukocytes is indicated (i.e. desirable from a therapeutic perspective).

25 For the purposes of the present invention, in such indications a *leukocyte deficiency* is deemed to have arisen.

30 It will therefore be understood that the leukocyte deficiencies in which the invention finds medical application encompass a very broad spectrum of diseases, syndromes, disorders, conditions and infections. For example, it will be appreciated that a leukocyte deficiency, in the special, broad sense defined above, can arise in circumstances where an individual has acquired a disease, syndrome, disorder, condition or infection involving leukocyte dysfunction as well as in

circumstances where an individual has acquired a disease, syndrome, disorder, condition or infection in which the endogenous leukocyte component is seemingly normal but in which alteration, augmentation or stimulation of the normal endogenous leukocyte activity is nevertheless indicated/required.

5

For convenience, such diseases, syndromes, disorders, conditions or infections are collectively defined herein as *leukocytic deficiencies*.

The therapies in which the present invention finds application may be broken
10 down into two broad classes. In a first class, the processes of the invention are employed to create a leukocyte composition (e.g. forming part of a leukocyte cell bank) from a blood sample from a healthy individual donor. In such applications, the invention is used to create a cellular resource of healthy leukocytic tissue from an individual donor that can be restored to that donor individual should the
15 individual acquire a leukocytic deficiency at a later date.

In such therapies (referred to herein as *restorative autotransplantation*), the invention exploits the fact that many leukocytic deficiencies occur as part of a temporal sequence of events (which may or may not be causally interrelated), so
20 that the creation of a leukocyte cell bank at a point in time predating onset of the leukocytic deficiency constitutes a therapeutic resource which can later be used restoratively.

In a second broad class, the processes of the invention are employed to create a leukocyte composition from a blood sample from an individual donor suffering
25 from a leukocytic deficiency. The leukocyte composition is then treated *in vitro* and the treated composition transplanted back into the individual. The treatment applied to the leukocyte composition is such that, when they are reintroduced into the donor, the leukocytic deficiency is ameliorated or eliminated. A variation of
30 this approach involves the *in vivo* treatment of the individual's leukocytes prior to the blood-sampling step, and such approaches may involve further *in vitro* treatment after the sampling step.

In such therapies, (referred to herein as *remedial autotransplantation*), the invention exploits the fact that many leukocytic deficiencies can be overcome by treatments applied to only a small subset of the total leukocyte pool present in an individual. Such treatments are discussed in greater detail below, and include 5 genetic modification, cellular expansion, selective elimination of particular cell types and stimulation with certain molecules (e.g. cytokines).

The concept of restorative autotransplantation described above can be applied to all individuals, whether healthy or not, and irrespective of factors that might serve 10 as indicators of susceptibility to leukocytic deficiency (for example age, medical history, genetic background and lifestyle). However, it does permit the identification of a particular class of individuals for which the processes of the invention may be particularly advantageously applied, as described in more detail in section III (entitled "Selection of donor individuals"). Moreover, since the 15 leukocyte deficiencies as defined above and treatable according to the invention by restorative or remedial autotransplantation embrace an enormous variety of known diseases, these are discussed in greater detail in the following section XII (entitled "Exemplary indications").

20

XII. Exemplary indications

As mentioned in the preceding section, the therapeutic and prophylactic uses of the invention encompass a very broad spectrum of diseases, syndromes, disorders, conditions and infections.

25

Infections

The invention may find application in the treatment of various infections. In this case, the endogenous leukocyte activity may be normal (or responding normally) 30 but its alteration, augmentation or stimulation is nevertheless desirable. In others (such as HIV infection) the endogenous leukocyte activity is dysfunctional as a direct consequence of infection.

Infections which may be treated or prevented according to the invention include bacterial, fungal or viral infections, or infections by any other organism e.g. a protozoan, nematode, insect or other parasite.

- 5 A preferred application is the treatment of AIDS as a result of HIV infection. Here, CD4⁺ cells can be collected from an individual when healthy or non-infected, and stored for subsequent transplantation into said individual when HIV infection manifests itself or when AIDS develops, or CD4⁺ cell count falls etc. Such a procedure may be attractive to an individual with a life-style likely to place
10 them at risk from contracting HIV infection.

Cancers, leukaemias and sarcomas

- 15 The invention may find application in the treatment and prophylaxis of various malignancies: in general, any malignant or pre-malignant condition, proliferative or hyper-proliferative condition or any disease arising or deriving from or associated with a functional or other disturbance or abnormality in the cells or tissues of the body.
20 Therapy or prophylaxis of various forms of cancer represents a preferred embodiment of the invention, and the treatment or prophylaxis of any cancerous cells or tissues of the body is contemplated.

Thus, the invention is not limited to any one type of proliferative disease (e. g. 25 leukaemias, lymphomas, carcinomas or sarcomas), nor is it restricted to specific oncogenes or tumour-suppressor gene epitopes such as ras, myc, myb, fos, fas, retinoblastoma, p53 etc. or other tumour cell marker epitopes that are presented in an HLA class I antigen restricted fashion or other such way so as to be identifiable by a leukocyte. All cancers such as leukaemia, lymphoma, breast, stomach, colon, 30 rectal, lung, liver, uterine, testicular, ovarian, prostate and brain tumours such as gliomas, astrocytomas and neuroblastomas, sarcomas such as rhabdomyosarcomas and fibrosarcomas are included for the therapy or prophylaxis by the present invention.

Thus, the present invention finds application in the treatment or prophylaxis of breast cancer, colon cancer, lung cancer and prostate cancer. It also finds application in the treatment or prophylaxis of cancers of the blood and lymphatic systems (including Hodgkin's Disease, leukemias, lymphomas, multiple myeloma, and Waldenström's disease), skin cancers (including malignant melanoma), cancers of the digestive tract (including head and neck cancers, oesophageal cancer, stomach cancer, cancer of the pancreas, liver cancer, colon and rectal cancer, anal cancer), cancers of the genital and urinary systems (including kidney cancer, bladder cancer, testis cancer, prostate cancer), cancers in women (including breast cancer, ovarian cancer, gynecological cancers and choriocarcinoma) as well as in brain, bone carcinoid, nasopharyngeal, retroperitoneal, thyroid and soft tissue tumours. It also finds application in the treatment or prophylaxis of cancers of unknown primary site.

15

XIII. Posology

Those skilled in the art will be readily able to determine the amount of leukocyte composition to be autotransplanted in the medical applications according to the invention. It should be noted that as few as 0.01×10^8 (e.g. $1-10 \times 10^8$) mature lymphocytes (which can be derived from a single sample of approximately 100 ml of normal human blood) are sufficient to boost the immune system of a subject and hence may have a beneficial effect according to the autologous transplantation method of the invention. It should be noted that the removal of a unit of blood is commonplace with over three million units of blood being taken, for allografting, from individuals annually in the UK alone.

The leukocyte composition administered may be derived from a single blood sample, or may constitute a pool of leukocyte compositions derived from a plurality of different blood samples taken from a donor individual at different times. The leukocyte composition administered may constitute all or a fraction of the deposited material, but preferably constitutes only a fraction thereof in order that multiple dosing can be achieved, optionally following cellular expansion of

the residue (for example, T cell numbers may be increased by *in vitro* expansion using standard methods).

- In applications based on T-cell activity, the number of mature T-cells
- 5 administered is at least 0.01×10^8 , more preferably at least 0.1×10^8 , more preferably at least 1×10^8 (e.g. at least $1-10 \times 10^8$). The preferred ranges are 0.01×10^8 to 10^{10} mature T lymphocytes, such as 0.1×10^8 to 10^{10} , 1×10^8 to 10^{10} or 1×10^9 to 10^{10} mature T lymphocytes.
- 10 Thus, the mature T-cell sample acquired for autotransplantation is at least 0.01×10^8 , generally in the range of $10^8 - 10^{10}$ CD3⁺ mature T-cells, preferably $2 \times 10^8 - 10^{10}$, more preferably $3 \times 10^8 - 10^{10}$ CD3⁺ and even more preferably $4-5 \times 10^8 - 10^{10}$ CD3⁺ mature T-cells.
- 15 Conveniently, each sample prepared for autotransplantation contains 3×10^8 CD3⁺ mature T-cells, more preferably 5×10^8 and even more preferably 1×10^9 CD3⁺ mature T-cells. If sufficient resources of blood are available from an individual, even more preferably still $4-5 \times 10^9$ CD3⁺ mature T-cells or 10^{10} CD3⁺ mature T-cells may be used.
- 20 Preferably, the mature T-cell subpopulation sample acquired for autotransplantation which is CD3⁺ and CD8⁺ is at least 0.01×10^8 , generally in the range of $0.25 \times 10^8 - 0.25 \times 10^{10}$, and more preferably $0.5 \times 10^8 - 0.25 \times 10^{10}$, and even more preferably $0.75 \times 10^8 - 0.25 \times 10^{10}$, and even more preferably still $0.75 \times 10^8 - 0.25 \times 10^{10}$ or $1.00 - 1.25 \times 10^8 - 0.25 \times 10^{10}$. Specific CD3⁺ and CD8⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.2×10^8 , preferably 0.4×10^8 , or more preferably 1×10^8 , or still more preferably 2×10^8 , or more preferably 3×10^8 , or more preferably 5×10^8 . If sufficient resources from an individual are available, 1×10^9 , preferably 2×10^9 , 4×10^9 , or more preferably 1×10^{10} CD3⁺ and CD8⁺ cells may be used.
- 30 Preferably, the mature T-cell subpopulation sample acquired for autologous transplantation which is CD3⁺ and CD4⁺ is at least 0.01×10^8 , generally in the range of $0.1 \times 10^8 - 0.5 \times 10^{10}$, and more preferably $0.65 \times 10^8 - 0.5 \times 10^{10}$, and

even more preferably 0.85×10^8 - 0.5×10^{10} , and even more preferably still 1×10^8 - 0.5×10^{10} or $1.8 - 3.6 \times 10^8$ - 0.5×10^{10} . Specific CD3⁺ and CD4⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.2×10^{10} , preferably 0.3×10^8 , or more preferably 0.4×10^8 , 0.5×10^8 , 1×10^8 , 2×10^8 , 5 3×10^8 , 4×10^8 , or more preferably 5×10^8 . If sufficient resources from an individual are available, 1×10^9 , or more preferably 2×10^9 , or more preferably 1×10^{10} CD3⁺ and CD4⁺ cells may be used.

Preferably, the mature T-cell natural killer subpopulation sample acquired for autotransplantation which is CD3⁺ and CD16/56⁺ is at least 0.01×10^8 , generally in the range of 0.01×10^8 - 0.5×10^{10} , preferably 0.02×10^8 - 0.5×10^{10} , more preferably 0.03×10^8 - 0.5×10^{10} , and even more preferably still 0.5×10^8 - 0.5×10^{10} or $0.5-2 \times 10^8$ to 0.5×10^{10} . Specific CD3⁺ and CD16/56⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.01×10^8 , 0.2×10^8 , 0.3×10^8 , 0.5×10^8 , 1×10^8 , 2×10^8 , 3×10^8 , 5×10^8 , or more preferably, if sufficient resources are available, 1×10^9 , or more preferably 2×10^9 , or more preferably 1×10^{10} CD3⁺ and CD16/56⁺ cells may be used.

In addition, the mature lymphocyte cell sample may preferably include B cells, such as CD19⁺ B lymphocytes. The mature B-cell sample included in the T-cell sample may be at least 10^7 , 10^8 or 10^9 , generally in the range of 10^7 - 10^{10} mature B-cells and preferably 2×10^7 - 10^{10} mature B-cells, more preferably 3×10^7 - 10^{10} mature B-cells, and even more preferably $4-5 \times 10^7$ - 10^{10} mature B-cells.

25 Specific numbers of B-cells in autograft is conveniently of the order of 3×10^7 , preferably 5×10^8 , more preferably 1×10^8 mature B-cells, and even more preferably still $4-5 \times 10^9$ or 10^{10} mature B-cells.

In addition, the lymphocyte cell sample may preferably include dendritic cells. 30 The dendritic cell sample may be at least 10^7 , 10^8 or 10^9 in number, and generally in the range of 10^7 - 10^{10} dendritic cells and preferably 2×10^7 - 10^{10} cells, more preferably 3×10^7 - 10^{10} cells, and even more preferably $4-5 \times 10^7$ - 10^{10} cells.

Specific numbers of dendritic cells in an autograft is conveniently of the order of 3

$\times 10^7$, preferably 5×10^8 , more preferably 1×10^8 , and even more preferably still $4-5 \times 10^9$ or 10^{10} mature B-cells.

5

XIV. Equivalents

The foregoing description details presently preferred embodiments of the present invention which are therefore to be considered in all respects as illustrative and not restrictive. Those skilled in the art will recognize, or be able to ascertain,
10 using no more than routine experimentation, many equivalents, modifications and variations to the specific embodiments of the invention described specifically herein. Such equivalents, modifications and variations are intended to be (or are) encompassed in the scope of the following claims.

CLAIMS:

1. A process for producing a leukocyte composition for autotransplantation comprising the steps of:
 - 5 (a) providing an isolated blood sample from a donor individual;
 - (b) selectively separating and collecting leukocytes from the sample using a leukapheresis device.
- 10 2. The process of claim 1 for producing a leukocyte composition for restorative autotransplantation, further comprising the steps of:
 - (c) rendering the collected leukocytes dormant (e.g. by cryogenic preservation); and optionally
 - (d) revitalizing the dormant leukocytes (e.g. by thawing and/or dilution).
- 15 3. The process of claim 1 for producing a leukocyte composition for remedial autotransplantation, further comprising the steps of:
 - (c) treating the collected leukocytes; and optionally
 - (d) rendering the treated leukocytes dormant (e.g. by cryogenic preservation).
- 20 4. The process of claim 3 further comprising the step of:
 - (e) revitalizing the dormant treated leukocytes (e.g. by thawing and/or dilution).
- 25 5. The process of claim 2 for producing a leukocyte cell bank, wherein the process is applied iteratively to a series of blood samples from different donor individuals to produce a plurality of dormant (e.g. cryogenically preserved) leukocyte compositions, the process further comprising the step of retrievably depositing the dormant leukocytes for later autotransplantation.
- 30 6. A system (e.g. a closed system) for collecting an isolated blood sample from an individual comprising a sample vessel and a leukapheresis tubing set.
7. The system of claim 6 wherein the tubing set comprises one or more (e.g. three) leukocyte collection vessel(s).

8. The system of claim 6 or claim 7 wherein the tubing set further comprises a blood processing vessel (e.g. a centrifuge loop).
- 5 9. The system of any one of claims 6 to 8 wherein the tubing set further comprises a vessel for residual blood from which the leukocytes have been removed.
- 10 10. The system of any one of claims 6 to 9 further comprising a needle for conducting a blood sample from the individual into the sample vessel.
11. Apparatus for selectively separating and removing leukocytes from an isolated blood sample from an individual comprising a leukapheresis device loaded with the collection system of any one of claims 6 to 10.
- 15 12. A leukocyte composition obtainable (or obtained) by the process of any one of claims 1 to 5.
13. A leukocyte cell bank obtainable (or obtained) by the process of claim 5.
- 20 14. The leukocyte composition of claim 12 for use in therapy or prophylaxis.
15. Use of the leukocyte composition of claim 12 for the manufacture of a medicament for use in autotransplantation (e.g. in restorative or remedial autotransplantation).

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